Kidney International, Vol. 57 (2000), pp. 787–793

Pathogenesis of Dent's disease and related syndromes of X-linked nephrolithiasis

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Pathogenesis of Dent's disease and related syndromes of X-linked nephrolithiasis. Renal stone disease, which affects 12% of males and 5% of females by the seventh decade, occurs as an inherited disorder in 45% of patients and is most commonly associated with hypercalciuria. The biochemical basis for hereditary nephrolithiasis and hypercalciuria is unknown, and this has therefore been investigated by a "positional cloning" approach. As a first step in this approach, the chromosomal locations of two disorders referred to as Dent's disease and X-linked recessive nephrolithiasis (XRN) were determined. These two disorders, which represent unusual forms of the renal Fanconi syndrome, are characterized by a low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis and renal failure. An X-linked inheritance for XRN was established by studies of a North American kindred, and a similar inheritance for Dent's disease was indicated by the observation of a greater disease severity in males and an absence of male-to-male transmission in five British families. X-linked polymorphic genetic markers were used in linkage studies of these families, and the genes causing Dent's disease and XRN were mapped to Xp11. In addition, in one family with Dent's disease, a microdeletion involving the DNA probe M27β was identified. This microdeletion was further characterized by using yeast artificial chromosomes (YACs) and its size was estimated to be 515 Kb. A search for renal-expressed genes from this region identified a novel gene encoding a chloride channel (*CLCN5*) with similarities to a family of voltage-gated chloride channels. Molecular genetic studies of *CLCN5* demonstrated that mutations, which resulted in a functional loss, were associated with Dent's disease and XRN. In addition, such *CLCN5* mutations that would result in a functional loss have also been demonstrated in Japanese children with idiopathic low molecular weight proteinuria, hypercalciuria and nephrocalcinosis, and an Italian kindred with X-linked recessive hypophosphatemic rickets (XLRH) and hypercalciuria. Thus, four hereditary disorders of nephrolithiasis are due to mutations of the novel chloride channel, *CLCN5*.

Renal stone disease (nephrolithiasis and nephrocalcinosis), which affects 12% of males and 5% of females by the age of 70 years [1, 2], occurs as an inherited disorder in 10% to 45% of patients [2, 3] and is most commonly associated with hypercalciuria [4]. The inheritance of nephrolithiasis and hypercalciuria has been established to be either autosomal dominant or X-linked in some families [3–9]. The biochemical basis for hereditary nephrolithiasis and hypercalciuria is unknown, and this has therefore been investigated by a "positional cloning" approach [10]. The first step in this approach is represented by determining the chromosomal locations of hereditary nephrolithiasis disorders, and then isolating the genes from the critical region. This approach has defined the molecular basis for four diseases of hereditary nephrolithiasis: Dent's disease, X-linked recessive nephrolithiasis (XRN), X-linked recessive hypophosphatemic rickets (XLRH), and the idiopathic low molecular weight proteinuria of Japanese children (JILMWP) [7, 8, 11–14]. The positional cloning studies resulted in the identification of a voltage-gated chloride channel, *CLCN5*, gene [15]; the progress in these studies will be reviewed.

DENT'S DISEASE AND RELATED SYNDROMES

Four disorders of hereditary hypercalciuric nephrolithiasis (kidney stones), referred to as Dent's disease [8, 9], XRN [5, 13], XLRH [7], and JILMWP [14], have been reported to be due to mutations of the X-linked renal specific voltage-gated chloride channel, *CLCN5*, gene (Table 1) [11, 12, 16–19]. All four of these diseases have features in common, and they represent renal tubular disorders that are characterized by low molecular weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis, nephrolithiasis and renal failure. In addition, other renal proximal tubular defects, which include aminoaciduria, phosphaturia, glycosuria, kaliuresis, uricosuria and an acquired impairment of urinary acidification, may also occur [5, 7, 9, 14]. However, there are differences between these disorders. For example, rickets has been a particular feature of Dent's disease and XLRH, but not XRN or JILMWP, and severe renal failure has been a feature of Dent's disease and XRN [5, 7–9, 13,

Key words: chloride channel gene, hypercalciuria, low molecular weight proteinuria, kidney stones.

Table 1. Mammalian voltage-gated chloride channel (*CLC*) genes and disease associations

Chloride channel	Chromosomal location	Function	Tissue distribution	Disease association
CLC-1	7q35	Voltage stabilization	Skeletal muscle	Thomsen's myotonia, Becker myotonia, Arrested Development of Righting (ADR) in mouse
CLC-2	3q26-3q28	Cell volume regulation	Ubiquitous	—
CLC-3	4	?	Multiple	—
CLC-4	Xp22.3	?	Muscle, brain, heart	—
CLC-5	Xp11.22	Endosomal chloride transport	Kidney (predominantly)	Dent's disease (nephrolithiasis)
CLC-6	1p36	?	Multiple (e.g. brain, testes, muscle, kidney)	—
CLC-7	16p13	?	Multiple (e.g. brain, testes, muscle, kidney)	—
CLC-Ka	1p36	Concentration gradient in counter-current mechanism	Kidney	Nephrogenic diabetes insipidus in mouse
CLC-Kb	1p36	Chloride reabsorption?	Kidney	Bartter's syndrome

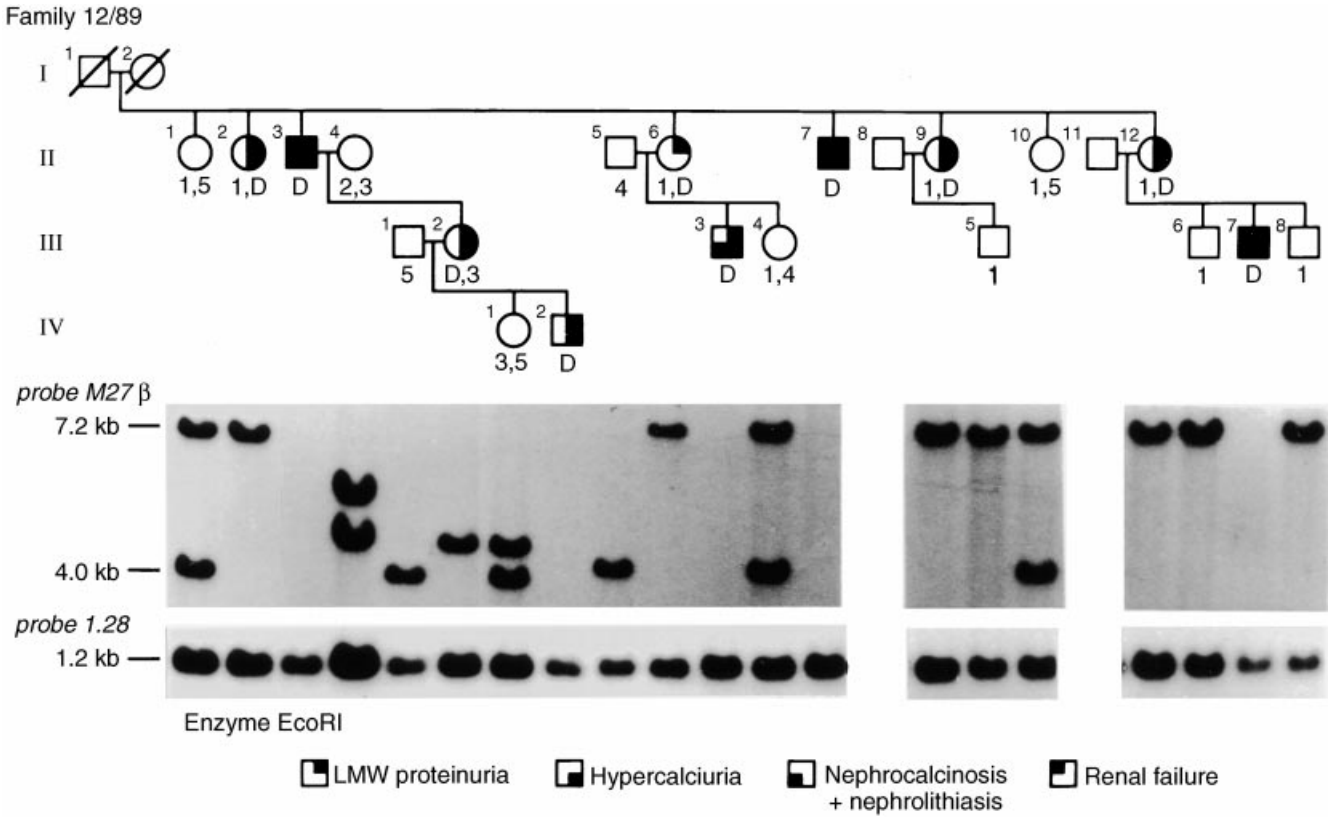


Fig. 1. Segregation of Dent's disease with a microdeletion detected by M27β. Probe M27β, which defines the locus DXS255 and has been localized to Xp11.22, hybridizes to EcoR1 fragments in the range of 3 to 7 kb in normal individuals, and heterozygosity in females exceeds 90%. Hybridization of the Southern blot (lower panel) from family 12/89 with probe M27β demonstrated an absence of signals in all the affected males (II.3, II.7, III.3, III.7 and IV.2) and only one fragment indicating hemizoygosity was detected in the affected females (II.2, II.6, II.9, II.12 and III.2). A control hybridization of this Southern blot with the probe L1.28, which defines the locus DXS7, yielded signals from all the lanes and demonstrated the presence of DNA in each lane. Thus, a microdeletion involving M27β is associated with Dent's disease in family 12/89, and this maps Dent's disease to Xp11.22. (Reproduced with permission from Pook et al, *Human Molecular Genetics* 2:2129–2134, 1993) [8].

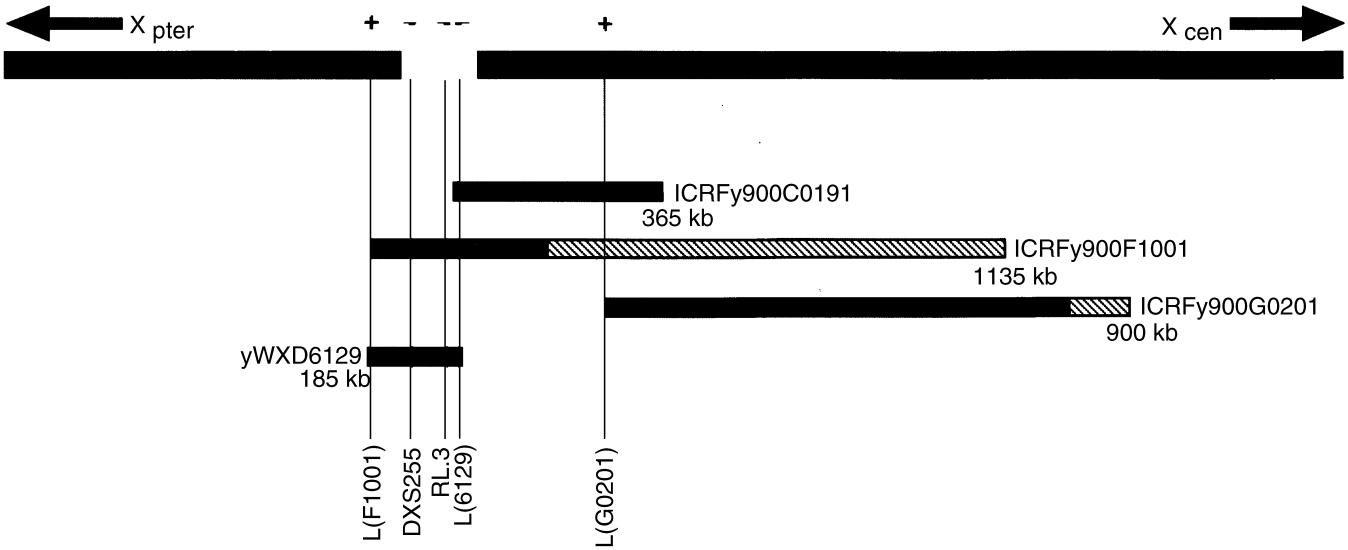
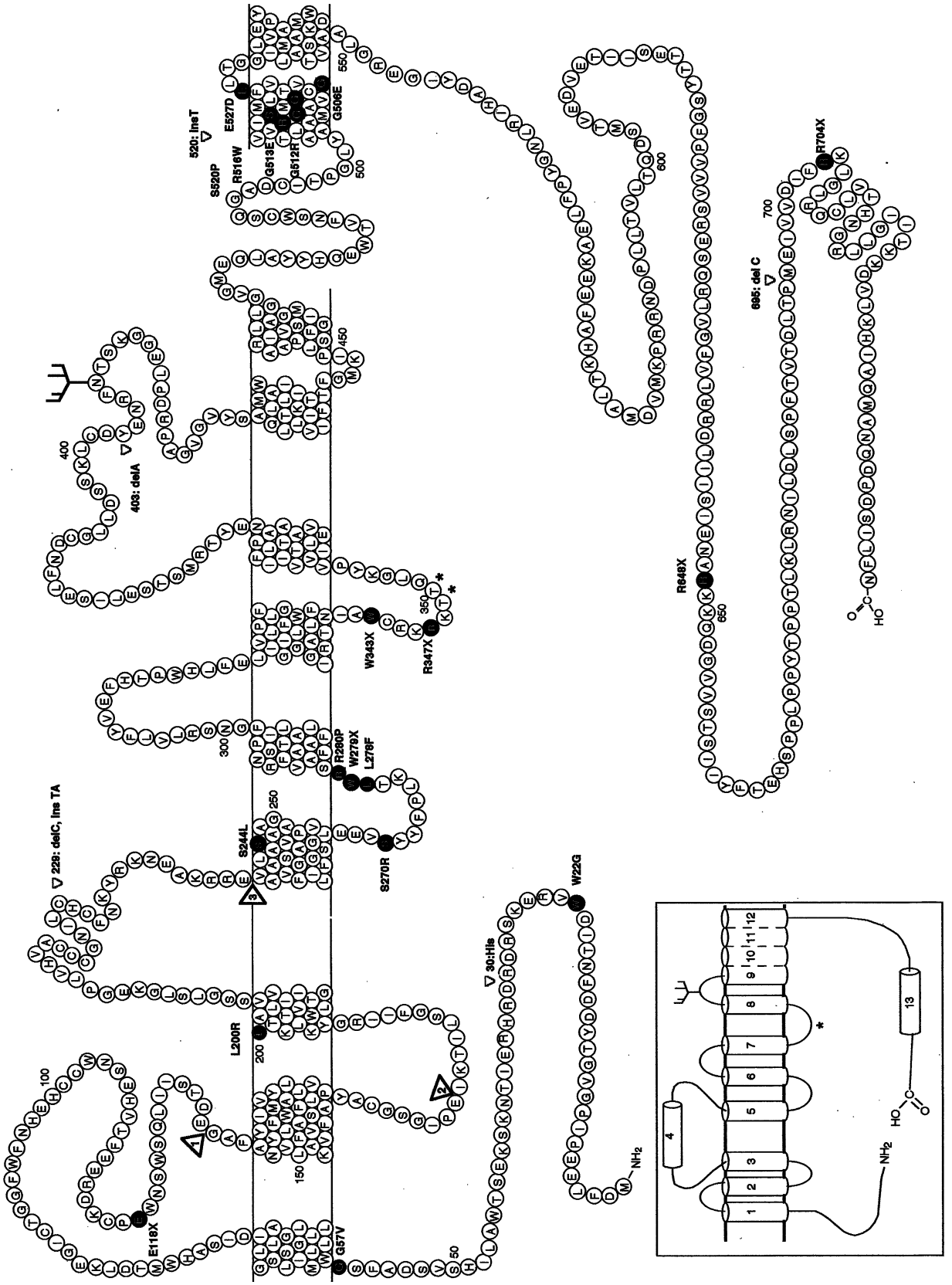


Fig. 2. Deletion mapping in a male patient with Dent's disease. The DXS255 (M27β) locus was deleted in the patient (II.3, family 12/89, Fig. 1) with Dent's disease and a yeast artificial chromosome (YAC) containing DXS255 was isolated. The YAC was 185 kb in size and the telomeric sequence, L(F1001), which was approximately 50 kb from DXS255, was present, while the centromeric sequence, L(6129), was absent. By using the centromeric YAC sequence, additional YACs were isolated and their terminal sequences similarly mapped with respect to the deletion. A YAC contig was established and the size of the microdeletion was revealed to be approximately 515 kb. Use of the 185 kb YAC as a hybridization probe to screen a renal cDNA library helped to isolate a novel gene, clone RL.3, which encoded a renal chloride channel CLC-5 (Fig. 3). (Reproduced with permission from Thakker, *Acta Nova Leopoldina* 302:23–33, 1997 [43]).

14]. Dent's disease, XRN and XLRH were mapped to Xp11.22 [7, 8, 13], and a microdeletion in one patient with Dent's disease (Fig. 1) facilitated the isolation and characterization (Fig. 2) of a renal chloride channel gene, *CLCN5* [8, 20, 21]. DNA sequence analyzes have detected different *CLCN5* mutations (Fig. 3), which consist of nonsense, missense, splice site, insertional and deletional mutations, in patients with these four hypercalciuric nephrolithiasis disorders [11, 12, 16–19], together with another form of a hereditary renal tubular acidosis [22], thereby establishing its causal role in these diseases [23, 24]. Heterologous expression of the wild-type *CLCN5* gene or its mutants in *Xenopus* oocytes also demonstrated that the wild-type channel, CLC-5, conducted outwardly directed chloride (Cl^-) currents that were either abolished or markedly reduced by the mutations [11, 12, 16]. The common genetic etiology of *CLCN5* mutations and the phenotypic similarities between all of these syndromes indicated that they were variants of one disorder, and it has been proposed to refer to them collectively as Dent's disease [12, 16].

VOLTAGE-GATED CHLORIDE CHANNEL (*CLC*) GENE FAMILY

The CLCs represent the most recently discovered of the three structurally defined classes of chloride channels [15]: (*1*) the extracellular ligand-gated (ELG) receptor chloride channels, for example, the glycine and gamma-amino-butyric acid ($GABA_A$) receptors [25, 26]; (*2*) the cystic fibrosis transmembrane conductance regulator (CFTR) Cl-channel [27]; and (*3*) the CLCs. The glycine and $GABA_A$ receptors, which probably have four transmembrane domains and may function as pentamers, conduct chloride ions and are mainly involved in neuronal inhibition. The CFTR, which is a member of the superfamily of adenosine 5′-triphosphate (ATP)-binding cassette (ABC) transporters and has 12 putative transmembrane domains arranged in two separate blocks with two cyclic nucleotide binding domains and a regulatory region, functions as a cyclic AMP (cAMP)-regulated chloride channel. The CLCs, which are structurally unrelated to the other ion channels and form the only known large family (Table 1) of Cl^- channels, consist of about 12 transmembrane domains [11, 20, 21, 28, 29]. The correct number and topology of these domains is being established, but domain 3 is likely to be a transmembrane domain [30], and recent studies of domain 4 using cysteine-scanning mutagenesis indicate that domain 4, which contains a conserved motif that is essential for anion selectivity, is likely to be involved in pore formation [31]. The first member, designated *CLC-0*, was cloned in 1990 from the electric organ of *Torpedo marmorata* [27], and nine different CLCs (CLC-1 to CLC-7, and CLC-Ka and CLC-Kb; encoded, respectively, by genes *CLCN1* to *CLCN7*, and *CLCNKa* and *CLCNKb*) have been identified in mammals [19, 28, 31]. Heterologous expression in *Xenopus* oocytes has revealed that the chloride


520: insT
S520P
R516W
E527D
G513E
G512R
G506E
403: delA
228: delC, ins TA
S244L
L200R
R280P
W279X
L278F
S270R
W343X
R347X
E118X
G57V
30:His
W22G
R648X
695: del C
R704X
NH2
HO

channels CLC-0, CLC-1, CLC-2 and CLC-5 conduct chloride currents that are outwardly rectifying and time-dependent, and with a conductivity sequence that prefers chloride to iodide [11, 12, 28–34]. These chloride channels are important for the control of membrane excitability, transepithelial transport and possibly regulation of cell volume [29]. CLCs are known to function as multimeric complexes, and recent studies have revealed that CLC-0 is a homodimer with two largely independent pores [33, 34]. The *CLC* genes are expressed in a variety of tissues (Table 1), and only *CLCN1*, *CLCN5* and *CLCNKb* have to date been reported to have disease associated mutations in humans [15]. Thus, mutations of *CLCN1* are associated with the myotonia disorders of Thomsen and Becker [35–37], mutations of *CLCN5* are associated with the hereditary nephrolithiasis disorders of Dent's disease [11, 12, 15], and mutations of *CLCNKb* are associated with a form of Bartter's syndrome [15, 38]. Recent studies of a mouse knockout model for *CLCNK1*, which is the murine homologue for the human *CLCNKa*, have demonstrated the occurrence of a form of nephrogenic diabetes insipidus (NDI) [39]. This results from a defect in the renal urinary concentrating mechanism and reveals an important role for *CLCNK1* in the countercurrent multiplication system of the inner medulla [39]. The human equivalent of this murine model for NDI remains to be identified.

ROLE OF *CLC-5* IN THE NEPHRON

The positional cloning [7, 8, 13, 20] and mutational analysis [11, 12, 16] of Dent's disease and its phenotypic variants indicated that *CLCN5* encodes a chloride channel, CLC-5, whose functional loss results in a generalized proximal tubular defect, that is, Fanconi syndrome, which is associated with the hypercalciuria and nephrolithiasis of Dent's disease [23]. However, the mechanisms whereby a loss of this renal chloride channel leads to hypercalciuria and the proximal tubular defects remains to be elucidated. The re-absorption of filtered protein occurs in the proximal tubule, whereas that of calcium occurs in proximal tubule, thick ascending limb of Henle's loop and the distal tubule. One possibility is that a loss of CLC-5 function in the proximal tubule may lead to a decrease in chloride re-absorption which in turn results in decreased calcium re-absorption [23, 40]. However, this does not explain the abnormal excretion of low molecular weight proteins, which are specifically absorbed in the proximal tubule by endocytosis and transported in an acidic vacuolar-lysosomal system [23, 41]. A loss of chloride channel function in this system would prevent the dissipation of the charge that is generated by the electrogenic $H^+-ATPase$ pump for the provision of the acidic environment. However, these possibilities needed to be explored, and the identification of the specific segments of the human nephron that express *CLCN5* represented an important step in this pathway towards further understanding the role and function of CLC-5 in the etiology of hypercalciuria and renal stones. These studies, which have detected the expression of CLC-5 by immunostaining, have revealed that CLC-5 expression occurs at multiple sites in the human nephron [42]. Immunohistochemistry studies have revealed that CLC-5 expression in the human nephron occurs in the epithelial cells lining the proximal tubules, the medullary thick ascending limbs of Henle's loop, and intercalated cells of the collecting ducts. Furthermore, studies of subcellular human kidney fractions indicated that CLC-5 is likely to be located in recycling endosomes, and that these may form part of the receptor-mediated endocytic pathway that reabsorbs low molecular weight proteins and albumin. Thus, CLC-5 dysfunction in the proximal tubule may result in low-molecular-weight proteinuria together with features of the Fanconi syndrome, and CLC-5 dysfunction in the thick ascending limb of Henle, which is the major site of calcium reabsorption, may result in hypercalciuria [42].

ACKNOWLEDGMENTS

I am grateful to: the Medical Research Council (MRC) in the United Kingdom for support; to my colleagues P.T. Christie, I.W. Craig, O. Devuyst, S. Fisher, B. Harding, T. Igarashi, T.J. Jentsch, S.E. Lloyd, S.H.S. Pearce, S.J. Scheinman, C. Wooding and O.M. Wrong for their collaborative efforts; and to Mrs. Sheila Kingsley for typing the manuscript.

Fig. 3. Schematic representation of a predicted topology of CLC-5. The mutations associated with Dent's disease, and its phenotypic variants are illustrated using the reported model [11] for CLC-5 topology. Every 50th amino acid of the 746-amino acid CLC-5 protein and the consensus phosphorylation (asterisk) and glycosylation (branch) sites at codons 349, 350 and 408, respectively, are indicated. The CLC-5 mutations are shown with the amino acid highlighted in black and the codon and substituted amino acid shown alongside. Arrows 1 and 2 delineate the amino acids predicted to be deleted by donor splice site mutations, and arrows 1 to 3 delineate those deleted in a Dent's disease family with a 2-kb intragenic deletion. The correct topology for the CLC channels is unknown and the predicted topology of the CLC-5 putative transmembrane domains (D1-D13) and is based upon a model [11] (inset) that places D4 extracellularly, and in which the hydrophobic core of the D9-D12 region crosses the membrane three or five times and contains a hydrophilic region (codons 481–502), the precise location of which remains unknown. More recent studies indicated that D4 is likely to be involved in pore formation [31]. More than 40 CLCN5 mutations have been reported in patients with Dent's disease and its phenotypic variants. Such CLCN5 mutations have been demonstrated, by heterologous expression in *Xenopus* oocytes, to result in a functional loss of the channel CLC-5 [11, 12, 16].

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REFERENCES

1. Consensus Conference: Prevention and treatment of kidney stones. *JAMA* 260:977–981, 1988
2. Smith LH: The medical aspects of urolithiasis: An overview. *J Urol* 141:707–710, 1989
3. Favus MJ: Familial forms of hypercalciuria. *J Urol* 141:719–722, 1989
4. Coe FL, Parks JH, Asplin JR: The pathogenesis and treatment of kidney stones. *N Engl J Med* 327:1141–1152, 1992
5. Frymoyer PA, Scheinman SJ, Dunham PB, Jones DB, Hueber P, Schroeder ET: X-linked recessive nephrolithiasis with renal failure. *N Engl J Med* 325:681–686, 1991
6. Furuse A, Futagoishi Y, Karashima S, Hattori S, Matsuda I: Familial progressive renal tubulopathy. *Clin Nephrol* 37:192–197, 1992
7. Bolino A, Devoto M, Enia G, Zoccali C, Weissenbach J, Romeo G: Genetic mapping in the Xp11.2 region of a new form of X-linked hypophosphatemic rickets. *Eur J Hum Genet* 1:269–279
8. Pook MA, Wrong OM, Wooding C, Norden AGW, Feest TG, Thakker RV: Dent's disease, a renal Fanconi syndrome with nephrocalcinosis and kidney stones, is associated with a micro–deletion involving DXS255 and maps to Xp11. *Hum Mol Genet* 2:2129–2134, 1993
9. Wrong OM, Norden AGW, Feest TG: Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. *Quart J Med* 87:473–493, 1994
10. Collins FS: Positional cloning: Let's not call it reverse any more. *Nat Genet* 1:3–6, 1992
11. Lloyd SE, Pearce SHS, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SPA, Wrong OM, Jentsch TJ, Craig IW, Thakker RV: A common molecular basis for three inherited kidney stone diseases. *Nature* 379:445–449, 1996
12. Lloyd SE, Pearce SHS, Gunter W, Kawaguchi H, Igarashi T, Jentsch TJ, Thakker RV: Idiopathic low molecular weight proteinuria associated with hypercalciuric nephrocalcinosis in Japanese children is due to mutations of the renal chloride channel (CLCN5). *J Clin Invest* 99:967–974, 1997
13. Scheinman SJ, Pook MA, Wooding C, Pang JT, Frymoyer PA, Thakker RV: Mapping the gene causing X-linked recessive nephrolithiasis to Xp11.22 by linkage studies. *J Clin Invest* 91:2351–2357, 1993
14. Igarashi T, Hayakawa H, Shiraga H, Kawato H, Yan K, Kawaguchi H, Yamanaka T, Tsuchida S, Akagi K: Hypercalciuria nephrocalcinosis in patients with idiopathic low molecular weight proteinuria in Japan: Is this identical to Dent's disease in the United Kingdom? *Nephron* 69:242–247, 1995
15. Thakker RV: Chloride channels cough up. *Nat Genet* 17:125–127, 1997
16. Lloyd SE, Gunter W, Pearce SHS, Thomson A, Bianchi ML, Bosio M, Craig IW, Fisher SE, Scheinman SJ, Wrong O, Jentsch TJ, Thakker RV: Characterisation of renal chloride channel, CLCN5, mutations in hypercalciuric nephrolithiasis (kidney stones) disorders. *Human Mol Genet* 6:1233–1239, 1977
17. Akuta N, Lloyd SE, Igarashi T, Shiraga H, Matsuyama T, Yokoro S, Cox JPD, Thakker RV: Mutations in CLCN5 in Japanese children with idiopathic low molecular weight proteinuria, hypercalciuria and nephrocalcinosis. *Kidney Int* 52:911–916, 1997
18. Oudet C, Martin-Coignard D, Pannetier S, Praud E, Champion G, Hanauer A: A second family with XLRH displays the mutation S244L in the CLCN5 gene. *Hum Genet* 99:781–784, 1997
19. Nakazato H, Hattori S, Furuse A, Kawano T, Karashima S, Tsuruta M, Yoshimutao T, Endo F, Matsuda I: Mutations in the CLCN5 gene in Japanese patients with familial idiopathic low-molecular-weight proteinuria. *Kidney Int* 52:895–900, 1997
20. Fisher SE, Black GCM, Lloyd SE, Wrong OM, Thakker RV, Craig IW: Isolation and partial characterisation of a human chloride channel gene which is expressed in kidney and is a candidate for Dent's disease (an hereditary nephrolithiasis). *Hum Mol Genet* 3:2053–2059, 1994
21. Fisher SE, Van Bakel I, Lloyd SE, Pearce SHS, Thakker RV, Craig IW: Cloning and characterisation of ClC-5, the human kidney chloride channel gene implicated in Dent's disease (an X-linked hereditary nephrolithiasis). *Genomics* 29:598–606, 1995
22. Kelleher CL, Buckalew OM, Frederickson ED, Rhodes DJ, Conner DA, Seidman JG, Seidman CE: CLCN5 mutation Ser244-Leu is associated with X-linked renal failure without X-linked recessive hypophosphatemic rickets. *Kidney Int* 53:31–37, 1998
23. Wrong OM, Unwin R, Cohen E, Tanner M, Thakker RV: Unravelling of the molecular mechanisms of kidney stones. *Lancet* 348:1561–1565, 1996
24. Scheinman SJ: X-linked hypercalciuric nephrolithiasis: Clinical syndromes and chloride channel mutations. *Kidney Int* 53:3–17, 1998
25. Grenningloh G, Rienitz A, Schmitt B, Methfessel C, Zensen M, Beyreuther K, Gundelfinger D, Betz H: The strychnine-binding sub-unit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature* 328:215–220, 1987
26. Schofield JR, Darlison MG, Fujita H, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, Seeburg PH, Barnard EA: Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature* 328:221–227, 1987
27. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, Drumm ML, Ianuzzi MC, Collins FS, Tsui LC: Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. *Science* 245:1066–1073, 1989
28. Jentsch TJ, Steinmeyer K, Schwarz G: Primary structure of *Torpedo marmorata* chloride channel isolated by expression cloning in *Xenopus* oocytes. *Nature* 348:510–514, 1990
29. Jentsch TJ, Gunther W, Pusch M, Schwappach B: Properties of voltage-gated chloride channels of the *ClC* gene family. *J Physiol* 482:19S–25S, 1990
30. Schmidt-Rose T, Jentsch TJ: Transmembrane topology of a CLC chloride channel. *Proc Natl Acad Sci USA* 94:7633–7638, 1997
31. Fahlke C, Yu HT, Beck CL, Rhodes TH, George JRAL: Pore-forming segments in voltage-gated chloride channels. *Nature* 390:529–532, 1997
32. Brandt S, Jentsch TJ: CL-6 and CLC-7 are two novel broadly expressed members of the CLC chloride channel family. *FEBS Lett* 377:15–20, 1995
33. Ludewig U, Pusch M, Jentsch TJ: Two physically distinct pores in the dimeric CLC-0 chloride channel. *Nature* 383:340–343, 1996
34. Middleton RE, Pheasant DJ, Miller C: Homodimeric architecture of a CLC-type chloride ion channel. *Nature* 383:337–340, 1996
35. Koch MC, Steinmeyer K, Lorenz C, Ricker K, Wolf F, Otto M, Zoll B, Lehmann-Horn F, Grzeschik K-H, Jentsch TJ: The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science* 257:797–800, 1992
36. Steinmeyer K, Lorenz C, Pusch M, Koch MC, Jentsch TJ: Multimeric structure of CLC-l chloride channel revealed by mutations in dominant myotonia congenita (Thomsen). *EMBO J* 13:737–743, 1994
37. Meyer-Kleine C, Steinmeyer K, Ricker K, Jentsch TJ, Koch MC: Spectrum of mutations in the major human skeletal muscle chloride channel gene (*CLCN1*) leading to myotonia. *Am J Hum Genet* 57:1325–1334, 1995
38. Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonca E, Stone R, Shurman S, Nayir A, Alpay H, Aysin B, Rodriguez-Soriano J, Morales JM, Sanjad SA, Taylor CM, Pilz D, Brem A, Trachtman H, Griswold W, Richard GA, Johne Lifton RP: Mutations in the chloride channel gene, *CLCNKB*, cause Bartter's syndrome type III. *Nat Genet* 17:171–178, 1997

39. Uchida S: CLC chloride channels in the kidney. Basic science symposium. *J Am Soc Nephrol* 9:1–127, 1998
40. Hebert SC: Crystal clear chloride channels. *Nature* 379:398–399, 1996
41. Christensen E, Nielsen S: Structural and functional features of protein handling in the kidney proximal tubule. *Semin Nephrol* 11:414–439, 1991
42. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV: Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. *Hum Mol Genet* (in press)
43. Thakker RV: Renal chloride channel, CLCN5, mutations and hypercalciuric neprolithiasis disorders. *Acta Nova Leopoldina Nr* 302:23–33, 1997